

Appl. No. : 10/009,792
Filed : December 13, 2001

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph beginning at page 7, line 26, with the following amended paragraph:

Finally, the complete hG-CSF protein is prepared from the protein pool obtained from the transformed *E. coli* using a Ni-column and a protease: the said *E. coli* secretes hG-CSF fusion protein of which N-terminus is linked by an oligopeptide consisting of 13 amino acid residues including 6 consecutive histidine residues. The secreted hG-CSF fusion protein is isolated using a Ni-column to which 6 consecutive histidine residues present in the oligopeptide of the fusion protein can bind, and then the complete hG-CSF protein can be prepared from the hG-CSF fusion protein isolated above by treating a protease to get rid of the oligopeptide. Since the hG-CSF protein has to be non-susceptible to the protease employed, the C-terminal sequence of the oligopeptide should be selected to be cleaved off by the protease of which recognition sequences are not present in the hG-CSF protein. As an example, in the present invention, the C-terminal amino acid sequence of the oligopeptide was selected to be Ile-Glu-Gly-Arg (see SEQ ID NO: 28, which is residue numbers 10-13 of SEQ ID NO: 1), which is recognized and cleaved by Factor Xa, a protease not having recognition sequences in hG-CSF protein.

Please replace the paragraph beginning at page 11, line 28, with the following amended paragraph:

Plasmid p19CSFm was amplified by PCR using primer 8 and primer 2. The resulting PCR product was digested with *NdeI* and *BamHI*, cloned into the same site of pET21c, and then transformed into *E. coli* XL1-Blue. The transformed *E. coli* XL1-Blue were selected on the LB agar medium containing ampicillin(50µg/l) and the recombinant plasmid pEDCSFmII was obtained therefrom(see: Figure 6). Comparing with the sequence of the fragment in pEDCSFm,

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the nucleotide sequence of the fragment in pEDCSFmII was found to be identical in N-terminal portion of the gene, meanwhile, be different in 6 nucleotide residues overall, which give rise the changes in 5 codons(ACC CCC CTG GGC CCT ACT CCG TTA GGT CCA; SEQ ID NO: 29).

Please replace the paragraph beginning at page 17, line 2, with the following amended paragraph:

To examine if the secreted hG-CSF protein has been processed correctly, i.e., if the signal sequence has been removed correctly, hG-CSF fusion protein was isolated from the gel and its N-terminal amino acid sequence was determined to be N'-Ala-Gly-Pro-His-His-His-His-His-Ile-Glu-Gly-Arg-Thr-C' (SEQ ID NO: 30), which is in agreement with the deduced amino acid sequence of N-terminal portion of hG-CSF fusion protein, indicating that the hG-CSF fusion protein was successfully secreted from *E. coli*. Of two temperature conditions, all the transformants showed higher secretion efficiency and higher hG-CSF protein content at 30° than 37°. Of 5 strains of transformed *E. coli*, BL21(DE3) and MC4100 showed the highest production yield. Thus, *E. coli* MC4100 transformed with the recombinant plasmid pTHKCSFmII was named *E. coli* MC4100/pTHKCSFmI, which was deposited with the Korean Collection for Type Cultures(KCTC) affiliated to Korea Research Institute of Bioscience and Biotechnology(KRIBB), an international depository authority, under accession(deposition) No. KCTC 0754BP on Mar. 13, 2000.